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Original Research Article

Preparation of Nanomaterials from Suspension of *Plerotus ostreatus* and *Agaricus bisporus* for Testing against *Klebsiella pneumoniae* and *Proteus mirabilis*

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Abstract: The antimicrobial activity of suspensions extracted from two fungal species, *Agaricus bisporus* and *Pleurotus ostreatus*, and a date palm leaves extract after adding a nanomaterial made of $AgNO_3$ was tested against two Gram-negative bacteria, *Klebsiella pneumoniae* and *Proteus mirabilis.* The result showed that the highest antimicrobial activity was reported in the suspension extract from *Agaricus bisporus,* followed by the palm leaves extract. In contrast, the lowest antimicrobial activity was observed in the suspension extract from *Plerotus ostreatus*. The extracts used in this research had a significant effect in inhibiting and limiting bacterial growth, in addition to the effect played by fungal suspended.

Keywords: Nanomaterials, *Agaricus bisporus, Pleurotus ostreatus, Klebsiella pneumoniae, Proteus mirabilis*.

1 - INTRODUCTION

Klebsiella pneumoniae and *Proteus mirabilis* are common opportunistic pathogens associated with many types of infections, particularly urinary tract infections, wound infections, respiratory infections, and blood infections. These bacteria have a high potential for developing multidrug resistance, posing a growing threat to public health and reducing the effectiveness of conventional treatments. (Nordmann *et al*., 2009, Armbruster & Moblley. 2012). In light of the increasing challenges associated with antibiotic resistance, the need to explore effective and safe therapeutic alternatives has increased. Among these promising alternatives is the use of bio-extracts from microorganisms, particularly fungi. Fungal filtrates (solutions containing secondary compounds produced by fungi) have demonstrated antibacterial activity due to their content of active bioactive compounds such as phenols, alkaloids, and terpenes. (Rasooli, I., & Abyaneh. 2004, Duru *et al*., 2005). In parallel, nanomaterials, particularly metallic nanoparticles such as zinc oxide (ZnO) and silver oxide (AgNPs), have attracted widespread interest due to their unique physical and chemical properties that enable them to penetrate the bacterial cell wall and interact with its vital components, inhibiting or killing the bacteria. Therefore, this research aims to evaluate the inhibitory effect of selected fungal filtrates and some nanomaterials on the growth of Klebsiella pneumoniae and Proteus mirabilis strains, as part of efforts to develop alternative and effective therapeutic strategies against antibiotic-resistant bacteria (Emam *et al*., 2016, Khalil *et al*., 2017).

2 - MATERIAL & METHODS

2-1- Bacterial Isolates

Two isolates of *K. pneumoniae and p. mirabilis* (Gram-negative), *P. ostreatus* & *A. bisporus* suspension were obtained from clinical samples and supplied by Department of applied Biotechnology, College of Biotechnology, Al-Qasim Green University.

2-2-Characterization of Silver Nanoparticles

UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (CE7200), England) from 200-900 nm at a resolution of 2nm.

2-3- Plant Material Preparation

The plant material was collected from the apex of the date palm, which is a yellow layer similar to the straw color that gathers on the leaves of the growing apex of the date palm. After that, 20gm of Date palm top powdered found on leaves were taken and extracted with Soxhlet apparatus ethanol (70%) Within 24 hours, and then taking the extract and placed in a petri dish, and put in the oven at a temperature of: (40°C) within 48 hours, the result of the extract was stored at: (4°C) until use (Harborne, 1999).

2-4- Preparation of the Fungal Suspended

Potato Dextrose Broth medium was distributed in glass flasks according to their use, and closed with cotton plugs, were sterilized by the autoclave at a temperature of 121 ° C and a pressure of 15 pounds / inch² for 20 minutes. Then inoculated with a disc of diameter 1 cm per 100 ml of the medium with the fungus *P. ostreatus and A.bisporus*, and placed in the incubator at a temperature of 25 °C. Perforated glass bottles were used for this experiment, and after 28 days of preparation had passed, the suspension was filtered and used for application (Narasimhamurthy Konappa, *et al*., 2021)

2-5- Synthesis of Silver Nanoparticles

Aqueous solution of silver nitrate was prepared by adding 1mM of AgNO3 to 9 ml of filtrate at room temperature. The aqueous solution was mixed 10 ml of filtrate of the fungus *P. ostreatus and A. bisporus* at a temperature of 70 °C while stirring magnetically at 1000 rpm for 10 min. The bio-reduced aqueous component was used for the UV–Vis spectroscopy characterization (Fig. 1, 3)

2-6- Evaluation of Antifungal Activity

The silver nanoparticles synthesized using filtrate of the fungus *P. ostreatus* and *A. bisporus* was tested for antifungal activity by Poisoned food method (28) against different pathogenic bacteria *K. pneumoniae* and *p. mirabilis* the pure cultures of fungi were sub cultured on Mueller-Hinton agar. After incubation at37◦C for 1 days, the diameter of colony (inhibition zoon) was measured in millimeter.

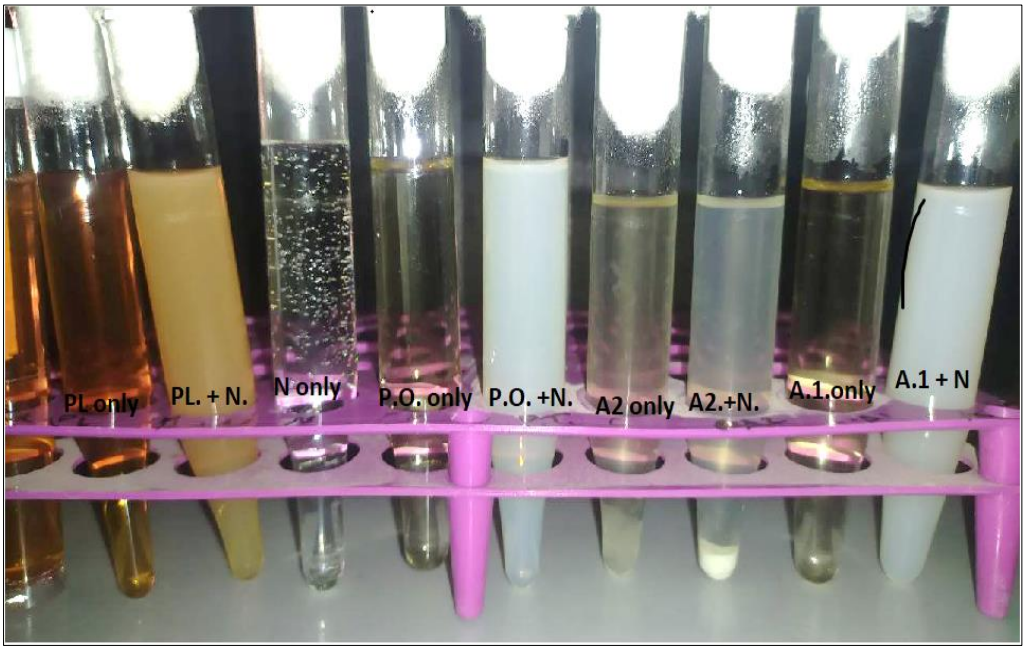

Fig. 1: Preparation of the samples and color change are evidence of the presence of nanomaterial

3 - RESULTS & DISCUSSION

The results of the study showed that all treatments, whether silver nitrate ($AgNO_3$) both *P ostreatus* and *A. bisporus*, alone or together, led to an inhibition of the growth of *K. pneumoniae and P. mirabilis*.(Fig 2) Silver nitrate demonstrated the highest antibacterial efficiency,(Fig. 4, 5) appeared wide inhibition zones due to its ability to penetrate the cell wall and interact with bacterial cell components, leading to their destruction. These results are consistent with what (Rai *et al*., 2009) indicated regarding the high effectiveness of silver nanoparticles as antimicrobials. As for fungi suspended, it demonstrated good inhibitory activity, with the effect being higher *on P. ostreatus* than on *A. bisporus.* This

is likely due to the filtrates containing active compounds such as phenols, terpenes, and alkaloids that inhibit bacterial growth, as confirmed by (Alves *et al*., 2012) in their review on the antibacterial properties of basidiomycetes. Remarkably, the combined treatment of silver nitrate and fungi suspended showed a synergistic effect, appeared to have the highest inhibition rates, indicating that the interaction between the physical mechanism of nanoparticles and the chemical mechanism of fungal compounds enhances the antibacterial efficacy, a phenomenon previously reported in studies such as Khalil *et al*., (2017). It was also observed that *K. pneumoniae* exhibited higher relative resistance compared to *P. mirabilis*, (Podschun and Ullmann 1998).

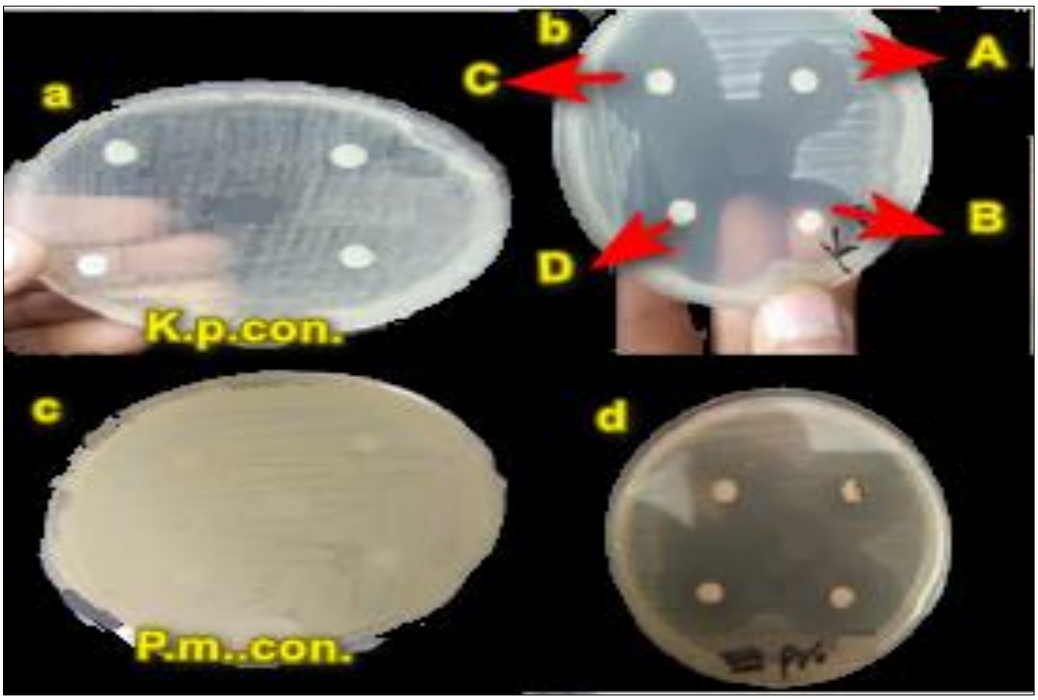

Fig. 2: Shows the percentage of inhibition of bacterial growth. (A) Fungal suspension for *P. ostreatus* with AgNO3, (B) Fungal suspension *A. bisporus* with AgNO3, (C) Palm leaf extract with AgNO3, (D) Palm stem extract with nanomaterial

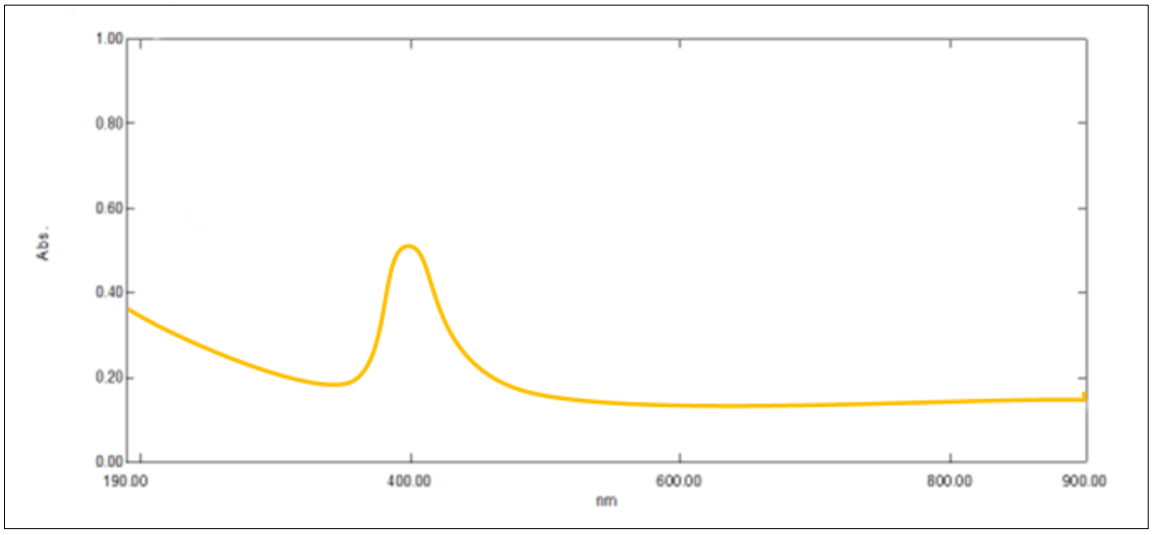

Fig. 3: UV-visible absorption spectrum of silver nanoparticle solution produced by (a) fungal suspension (*P. ostreatus A. bisporus*) +silver nitrate

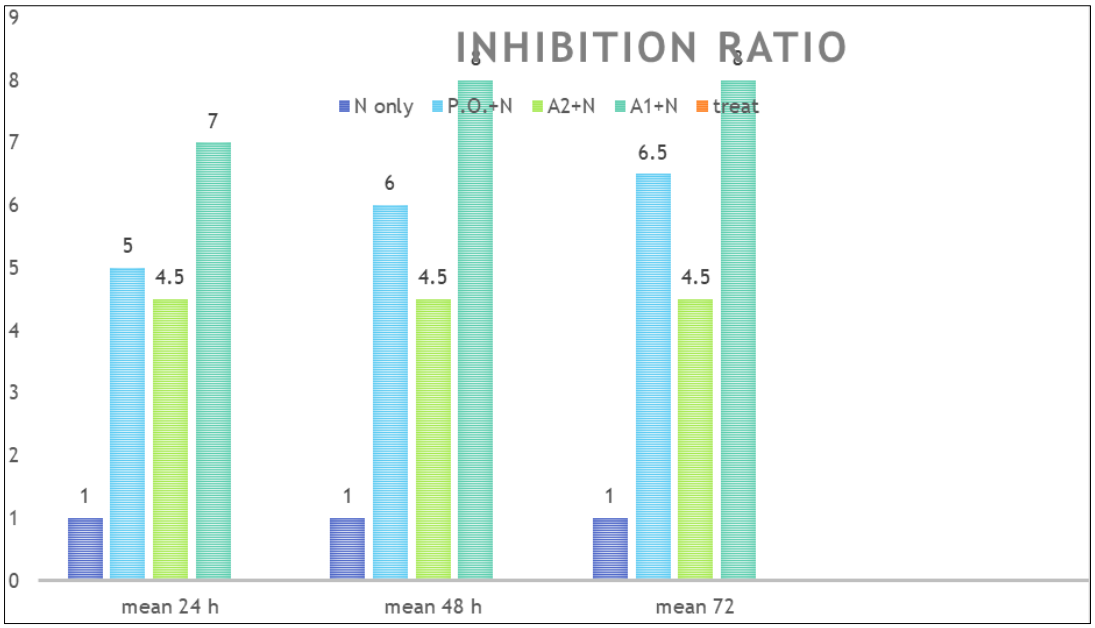

Fig. 4: Show the ratio of inhibition agonist *K. pneumoniae* in 24, 48, 72 hours

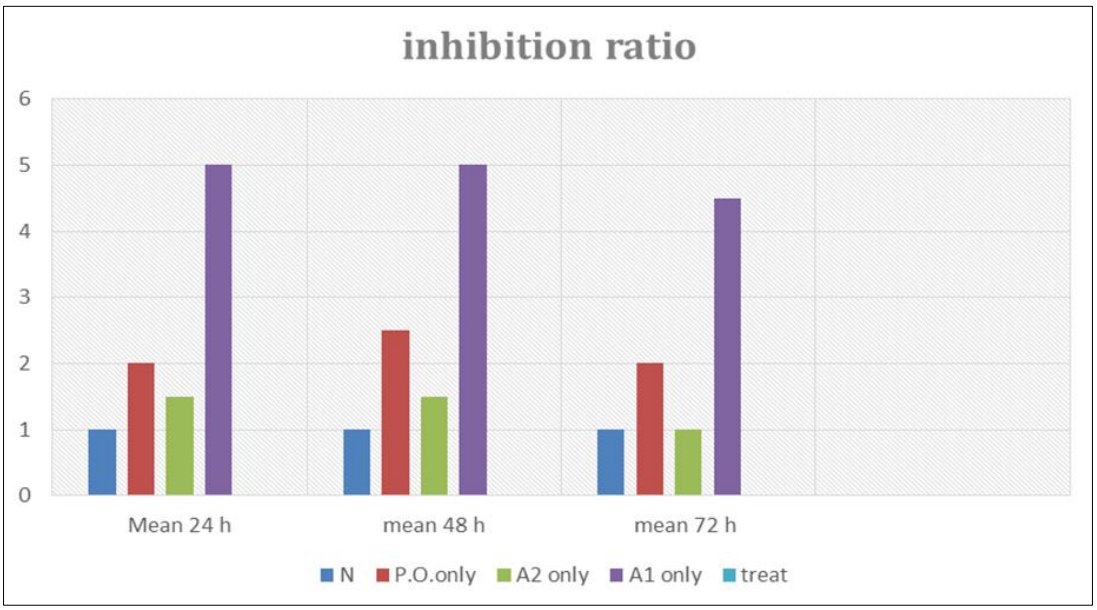

Fig. 5: Show the ratio of inhibition agonist *p. mirabilis* in 24, 48, 72 hours

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